Nanosphere

510(K) Summary

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The Summary for this 510(k) submission is submitted in accordance with the requirements of SMDA 1900 and CFR 807.92

510(k) Number:

K140083: Verigene® Enteric Pathogens Nucleic Acid Test (EP)

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Submitted by:

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Proprietary Names:

For the instrument:

Verigene® System

For the assay:

Verigene® Enteric Pathogens Nucleic Acid Test (EP)

Common Names:

For the instrument:

Bench-top molecular diagnostics workstation

For the assay:

Enteric Pathogens Nucleic Acid Test
Enteric Pathogens identification and differentiation system
Enteric assay
Enteric test

Regulatory Information:

Regulation section:

866. 3990 - Gastrointestinal microorganism multiplex nucleic acid-based assay

Classification:

Class II

Panel:

Microbiology (83)

Product Code(s):

PCH Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System
PCI Gastrointestinal Bacterial Panel Multiplex Nucleic Acid-based Assay System
OOI Real Time Nucleic Acid Amplification System

Other codes used by predicate device:

NSU Instrumentation for clinical multiplex test systems
JJH Clinical Sample Concentrator

Predicate Devices:

xTAG® Gastrointestinal Pathogen Panel (GPP) (K121894) (Luminex Molecular Diagnostics, Inc.)

Indications for Use:

The Verigene® Enteric Pathogens Nucleic Acid Test (EP) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria and genetic virulence markers from liquid or soft stool preserved in Cary-Blair media, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria:

- *Campylobacter* Group (comprised of *C. coli*, *C. jejuni*, and *C. lari*)
- *Salmonella* species
- *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*)
- *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*)
- *Yersinia enterocolitica*

In addition, EP detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2.

EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. EP also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens.

Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

EP results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative EP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

Technological Characteristics:

The Verigene Enteric Pathogens Nucleic Acid Test (EP) is a molecular assay which relies on detection of specific nucleic acid targets in a microarray format. For each of the bacterial nucleic acid sequences detected by EP, unique Capture and Mediator oligonucleotides are utilized, with gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides are covalently bound to the microarray substrate and hybridize to a specific portion of the nucleic acid targets. The Mediator oligonucleotides have a region which bind to a different portion of the same nucleic acid targets and also have a sequence which allows binding of a gold nanoparticle probe. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency and provide accurate detection of target capture.

The EP test is performed on the Verigene System, a "sample-to-result", fully automated, bench-top molecular diagnostics workstation. The System enables automated nucleic acid extraction from unformed stool specimens (liquid or soft) preserved in Cary-Blair media and detection of bacterial-specific target DNA. The Verigene System consists of two components: the Verigene Reader and the Verigene Processor *SP*.

The Reader is the Verigene System's user interface, which serves as the central control unit for all aspects of test processing, automated imaging, and result generation using a touch-screen control panel and a barcode scanner. The Verigene Processor *SP* executes the test procedure, automating the steps of (1) Sample Preparation and Target Amplification – cell lysis and magnetic bead-based bacterial DNA isolation and amplification, and (2) Hybridization–detection and identification of bacterial-specific DNA in a microarray format by using gold nanoparticle probe-based technology. Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of the trays and finally loads the specimen into the Test Cartridge for hybridization. Single-use disposable test consumables and a self-contained Verigene Test Cartridge are utilized for each sample tested with the EP assay.

To obtain the test results after test processing is complete, the user removes the Test Cartridge from the Processor *SP*, and inserts the substrate holder into the Reader for analysis. Light scatter from the capture spots is imaged by the Reader and intensities from the microarray spots are used to make a determination regarding the presence (Detected) or absence (Not Detected) of a bacterial nucleic acid sequence/analyte. This determination is made by means of software-based decision algorithm resident in the Reader.

Performance Data - Analytical Testing

Analytical Sensitivity / Limit of Detection (LoD)

Analytical sensitivity (LoD) of the EP test was determined for 16 strains of enteric pathogens, representing all seven (7) EP test reportable target analytes. The LoD was defined as the concentration at which the test produces a positive result at least 95% of the time. Serial dilutions of the strains were tested and the putative LoD confirmed with 20 replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, a further 20 replicates were performed at the next lower concentration until <95% was achieved. The LoDs for the 16 strains tested, and the corresponding LoD ranges for the EP test reportable target, are shown in the table below. Overall, the LoDs range from $4.10x10^3$ to $3.33x10^5$CFU/mL of stool.

Representative Organism Tested	***ATCC Source Number***	***Organism LoD*** *(CFU/mL)*	***Reportable Target***	***EP Test Target LoD*** *(CFU/mL Stool)*
Campylobacter jejuni subsp *jejuni*	43429	$3.70x10^4$	Campylobacter	$3.70x10^4$ - $1.11x10^5$
Campylobacter coli	43482	$1.11x10^5$		
Campylobacter lari	35222	$3.70x10^4$		
Salmonella enterica subsp *enterica* serovar *typhi*	9993	$3.33x10^5$	Salmonella	$3.33x10^5$
Salmonella enterica subsp *arizonae*	13314	$3.33x10^5$		
Shigella dysenteriae / Shiga Toxin 1	29026	$3.70x10^4$	Shigella, Stx1	$3.70x10^4$ - $1.11x10^5$
Shigella flexneri	25929	$1.11x10^5$	Shigella	
Shigella sonnei	29030	$3.70x10^4$		
Shigella boydii	12035	$1.11x10^5$		
Vibrio cholerae	39315	$1.11x10^5$	Vibrio	$3.70x10^4$ - $1.11x10^5$
Vibrio parahaemolyticus	49398	$3.70x10^4$		
Yersinia enterocolitica	700822	$3.33x10^5$	Yersinia enterocolitica	$1.11x10^5$ - $3.33x10^5$
	23715	$1.11x10^5$		
E. coli – Shiga Toxin 1	43890	$4.10x10^3$	Stx1	$4.10x10^3$ - $3.70x10^4$
E. coli – Shiga Toxin 2	BAA-176	$1.11x10^5$	Stx2	$3.70x10^4$ - $1.11x10^5$
E. coli – Shiga Toxin 1 / Shiga Toxin 2	43895	$3.70x10^4$		

Analytical Reactivity (Inclusivity)

Analytical reactivity of the EP test was demonstrated with a comprehensive panel of 111 clinically relevant bacterial strains representing temporal, geographical, and phylogenic diversity for each claimed target (see table below). For the Stx1 and Stx2 targets, Shiga toxin producing organisms tested included the vast majority of serotypes isolated in the U.S and those that are outbreak-related. All 111 strains generated the expected result when tested in triplicate at a concentration of three times LoD.

Reportable Target	***Total Number of Organisms/Strains Tested***	***Species Tested***	
		Name (No. of Strains)	*Total Number*
Campylobacter	15	*C. coli (5), C. jejuni subsp jejuni (4), C. jejuni subsp doylei (1), C. lari (5)*	3
Salmonella	31	*S. bongori (1), S. enterica subsp various (5), S. enterica subsp enterica serovar various (25)*	2
Shigella	20	*S. boydii (5), S. dysenteriae (5)*[a], *S. flexneri (5), S. sonnei (5)*	4
Vibrio	10	*V. cholerae (5), V. parahaemolyticus (5)*	2
Yersinia enterocolitica	7	*Y. enterocolitica (7)*	1
Shiga toxin 1	19	*S. dysenteriae (2)*[a], *E. coli (17)*[b]	2
Shiga toxin 2	16	*E. coli (16)*[b]	1

[a] Two (2) strains contain Stx1

[b] Five (5) strains contain both Stx1 and Stx2

Analytical Specificity (Cross-reactivity)

One-hundred and sixty-one (161) organisms, consisting of 135 bacterial organisms, 21 viruses, four (4) parasites and one (1) human cell line were tested with the EP test to determine analytical specificity (see table below). Eight (8) organisms, including Astrovirus and Sapovirus (2 strains), *Campylobacter hominis* and all four parasites were tested as genomic DNA/RNA. In addition, to rule out cross-reactivity between the analytes detected by the EP test, six organisms representing all of the EP test detected targets, were tested at elevated concentrations of 5×10^6 CFU/mL. The exclusivity of 15 species of *Vibrio* not associated with human infection, four (4) non-pathogenic strains of *Escherichia coli*, *Yersinia pestis*, and *Clostridium botulinum* were evaluated by *in silico* analysis alone.

All of the organisms tested yielded the expected "Not Detected" results, indicating that there was no cross-reactivity with the EP test, with the exception of *Campylobacter insulaenigrae* which yielded a single positive result (1/9) for "Campylobacter". *In silico* analysis also indicates a potential for low-level cross-reactivity. While *Campylobacter insulaenigrae* has been isolated primarily from marine mammals, in rare cases it may cause septicemia and gastroenteritis in humans.[1]

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[1] J Med Microbiol. 2007 Nov;56(Pt 11):1565-7.

Organisms Tested for Analytical Specificity

Bacterial Non-Test Panel Members

Genus	Species
Abiotrophia	*defectiva*
Acinetobacter	*baumannii*
	lwoffii
Acrobacter	*butzleri*
	cryaerophilus
Aeromonas	*allosaccharophila*
	bestiarum
	caviae
	encheleia
	enteropelogenes
	eucrenophila
	hydrophilia
	jandaei
	*salmonicida**
	veronii
Alcaligenes	*faecalis*
Bacillus	*cereus*
Bacteroides	*caccae*
	fragilis
	merdae
	stercoris
Candida	*albicans*
Cedecea	*davisae*
Citrobacter	*amalonaticus*
	freundii
	sedlakii
Clostridium	*bifermentans*
	bolteae
	butyricum
	difficile (2 strains)
	difficile, non-tox
	haemolyticum
	methylpentosum
	nexile
	novyi
	orbiscindens
	perfringens
	scindens
	septicum
	sordellii
	spiroforme
	sporogenes
Colinsella	*aerofaciens*
Desulfovibrio	*piger*
Edwardsiella	*tarda*
Enterobacter	*aerogenes*
Enterobacter	*cloacae*
Enterococcus	*faecalis*
	faecium

Genus	Species
Escherichia	*coli* (3 strains)
	coli (EAEC)
	coli (EPEC) (2)
	coli (ETEC) (2)
	fergusonii
	hermannii
Fusobacterium	*varium*
Helicobacter	*hepaticus*
	pylori (4 strains)
Klebsiella	*oxytoca*
	pneumoniae
Lactobacillus	*acidophilus*
	reuteri
	rhamnosus
Lactococcus	*lactis*
Leminorela	*grimontii*
Listeria	*gravi*
	monocytogenes
Morganella	*morganii*
Peptostreptococcus	*anaerobius*
Plesiomonas	*shigelloides*
Porphyromonas	*asaccharoluticus*
Prevotella	*melaniogenica*
Proteus	*mirabilis*
	vulgaris
	penneri
Providencia	*stuartii*
	alcalifaciens
	rettgeri
Pseudomonas	*aeruginosa*
	fluroescenes
	putida
	aeruginosa
Ruminococcus	*bromii*
Serratia	*liquefacians*
	marcescens
Staphylococcus	*aureus*
	epidermidis
Streptococcus	*agalactiae, O90R*
	dysgalactiae
	mutans

Parasites

Blastocystis	*hominis*
Cryptosporidium	*parvum*
Entamoeba	*histolytica*
Giardia	*lamblia*

Human Cell Line

Colon epithelial cells (colorectal adenocarcinoma)

* *Sub-species masoucida and sub-species salmonicida (2 strains)*

Bacterial EP Test Panel Members

Genus	Species
Campylobacter	*concisus*
	curvus
	fetus
	gracilis
	hominis
	hyointestinalis
	insulaenigrae
	lanienae
	mucosalis
	rectus
	showae
	sputorum
	upsaliensis
Vibrio	*alginolyticus*
	campbellii
	cincinnatiensis
	fluvialis
	furnissii
	harveyi
	metschnikovii
	mimicus
	tubiashii
	vulnificus (3 strains)
Yersinia	*aldovae*
	aleksiciae
	bercovieri
	frederiksenii
	intermedia
	kristensenii
	mollaretii
	pseudotuberculosis
	ruckeri
	rohdei

Viruses

Name	Serovar / Group
Adenovirus	Type 1/Group C
	Type 2/Group C
	Type 3/Group B1
	Type 4/Group E
	Type 5/Group C
	Type 14/Group B2
	Type 26/Group D
	Type 31/Group A
	Type 37/Group D
	Type 40/Group F
	Human 4
Astrovirus	-
Coxsackievirus B4	-
Cytomegalovirus	-
Echovirus 11	-
Enterovirus 68	-
Norovirus	Genogroup GI
	Genogroup GII
Rotavirus	Genogroup A
Sapovirus	

Microbial Interference

Two representative bacterial organisms detected by the EP test, *Campylobacter jejuni* and *Escherichia coli* (Shiga toxin 1), were evaluated for potential interference in the presence of 14 potentially interferent microorganisms not detected by the EP test, including *Bacteroides fragilis, Prevotella oralis, Prevotella melaninogenicus, Bifidobacterium bifidum, Clostridium perfringens, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Lactobacillus acidophilus, Staphylococcus aureus, Blastocystis hominis, Entamoeba histolytica,* and *Candida albicans*. These 14 microorganisms represent the most prevalent bacteria known to be present in the human colon and therefore are the most likely to be encountered in stool specimens tested with the EP test. These normal flora bacteria were tested at a concentration of 10^7 CFU/mL with the exception of the parasites *Blastocystis hominis* and *Entamoeba histolytica* which were tested at $9x10^6$ cells/mL and $7x10^5$ cells/mL respectively. No interference was observed with the EP test for any of the samples tested.

Interference (Exogenous Substances)

A comprehensive interfering substances study was performed to assess the potential inhibitory effect of endogenous and exogenous substances that can commonly be found in clinical stool specimens. Two organisms representative of the target analytes detected by the EP test, i.e., *Campylobacter jejuni* and *Escherichia coli* (Shiga toxin 1), were individually challenged with 22 potentially interfering substances (shown in the following table) at high, medically-relevant concentrations. None of the 22 substances tested showed any inhibitory effect on the detection of target enteric pathogens using the EP test.

Intralipid	Vaseline Original 100% Pure Petroleum Jelly
Cholesterol	Tums Antacid with Calcium Extra Strength 750
Whole Blood	Gaviscon Extra Strength Liquid Antacid
Mucus (Nasopharyngeal swab sample in UTM)	Mesalazine
Nystatin Suspension	Immodium® AD Anti-Diarrheal
Preparation H® Anti-itch Hydrocortisone 1%	Pepto-Bismol Max Strength
Desitin Maximum Strength Original Paste	MetronidazoleTopical Cream (0.75%)
Preparation H® Hemorrhoidal Ointment	Naproxen Sodium
Options Conceptrol®Vaginal Contraceptive Gel	Mucin from bovine submaxillary glands, Type I-S
Wet Ones®Antibacterial Hand Wipes	Barium Sulfate
K-Y®Personal Lubricant Jelly	Amoxicillin (Antibiotic)

Carryover / Cross-contamination

The potential for carryover and cross-contamination of the EP test on the Verigene system was assessed by alternately testing six representative high positive enteric pathogen samples (*Yersinia enterocolitica, Shigella dysenteriae* / Stx1, *Escherichia coli* / Stx2, *Salmonella enterica enterica, Campylobacter jejuni* , and *Vibrio cholera*) at $5x10^6$ CFU/mL, followed by testing a negative stool sample. The high-titer sample was alternated with the negative sample three times on six unique Verigene *SP* Processors. No carryover or cross-contamination was observed.

Competitive Inhibition

Binary combinations of all six of the EP test panel organisms representing all possible dual infections were evaluated, using simulated samples prepared in Negative Stool Matrix (NSM), with one panel organism present at a Low Positive titer (3x LoD) and a second organism present at a High Positive titer (> 10^6 CFU/mL stool). The performance of the EP test was evaluated with each of the 30 unique sample combinations tested in replicates of three (3). The EP test correctly detected both bacterial target organisms present in the co-infection combinations tested with one exception. For the Low Titer *Campylobacter coli* and High Titer *E. coli*/Stx2 sample, the EP test did not detect *Campylobacter* in one of the three replicates, although Shiga Toxin 2 was correctly identified in all cases. However, repeat testing indicated that this observation was not indicative of competitive inhibition.

Cutoff Verification

Target mean intensity values observed with the EP test were examined for the testing of the sixteen bacterial samples used to establish the Limit of Detection of the assay. In addition, the cut-off data set included the test results of three negative control samples. With replicates of 20 for each sample and ten target spot groups evaluated per test, a total of 3800 data points (1120 expected positive) were assessed to verify the assay cut-off.

Precision

The precision study was conducted in-house by Nanosphere, during which a fourteen-member simulated sample panel was tested daily in duplicate by two (2) operators for four (4) non-consecutive days for a total of sixteen (16) tests per sample. In total, the study yielded 224 test results. The fourteen (14) sample panel comprised six (6) different strains at two (2) different concentrations (12 positive samples) and two (2) negative samples (Negative Stool Matrix and *Clostridium difficile*). This panel included for each strain, a "Low Positive" sample (defined as approximately 1-2x LoD), which would be expected to produce a positive result approximately 95% of the time, and a "Moderate Positive" sample (defined as approximately 2-5x LoD), which would be expected to yield a positive result approximately 100% of the time. Results are summarized below.

Sample	*EP Test Expected Call*	*Conc.*	*Agreement w/ Expected Result (95 % CI)*[a]	*Sample*	*EP Test Expected Call*	*Conc.*	*Agreement w/ Expected Result (95 % CI)*[a]
Escherichia coli/Stx2	E. coli Stx2	Moderate	100% 16/16 (79.4%-100%)	*Campylobacter jejuni*	Campylobacter	Moderate	100% 16/16 (79.4%-100%)
		Low	100% 16/16 (79.4%-100%)			Low	100% 16/16 (79.4%-100%)
Salmonella enterica	Salmonella	Moderate	100% 16/16 (79.4%-100%)	*Vibrio parahaemolyticus*	Vibrio	Moderate	100% 16/16 (79.4%-100%)
		Low	93.8% 15/16[b] (69.8%-99.8%)			Low	100% 16/16 (79.4%-100%)
Shigella dysenteriae Stx1	Shigella Stx1	Moderate	100% 16/16 (79.4%-100%)	Negative Stool Matrix	All Targets Not Detected	NA	100% 16/16 (79.4%-100%)
		Low	100% 16/16 (79.4%-100%)	*Clostridium difficile*	All Targets Not Detected	NA	100% 16/16 (79.4%-100%)
Yersinia enterocolitica	Y. enterocolitica	Moderate	100% 16/16 (79.4%-100%)				
		Low	100% 16/16 (79.4%-100%)				

[a] 95% Two-sided Exact Binomial Confidence Interval calculation using the exact Clopper-Pearson method.
[b] One sample called "Salmonella" and "Stx2."

Performance Data - Clinical Testing

Reproducibility

The inter-laboratory reproducibility of the EP test was determined by conducting a reproducibility study at three external sites. Fourteen (14) unique samples were tested daily in triplicate by two (2) operators for five (5) non-consecutive days at three (3) sites for a total of ninety (90) tests per sample. The study tested a total of 1260 samples. The fourteen (14) sample panel was the same panel described previously for the precision study comprising six (6) different strains at two (2) different concentrations (12 positive samples) and two (2) negative samples (Negative Stool Matrix and *Clostridium difficile*). The results of the Reproducibility Study are provided in the table below.

Sample	*Expected Call*	*Conc.*	*Total Agreement with Expected Result (95 % CI)*		
			Site 1	*Site 2*	*Site 3*
Escherichia coli/Stx2	E. coli Stx2	*Moderate*	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
		Low	29/30 96.7% (82.8-99.9)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
Salmonella enterica	Salmonella	*Moderate*	28/30 93.3% (77.9-99.2)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
		Low	26/30 86.7% (69.3-96.2)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
Shigella dysenteriae/Stx1	Shigella Stx1	*Moderate*	30/30 100% (88.4-100)	28/30 93.3% (77.9-99.2)	30/30 100% (88.4-100)
		Low	29/30 96.7% (82.8-99.9)	29/30 96.7% (82.8-99.9)	28/30 93.3% (77.9-99.2)
Yersinia enterocolitica	Y. enterocolitica	*Moderate*	29/30 96.7% (82.8-99.9)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
		Low	28/30 93.3% (77.9-99.2)	27/30 90.0% (73.5-97.9)	25/30 83.3% (65.3-94.4)
Campylobacter jejuni	Campylobacter	*Moderate*	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
		Low	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
Vibrio parahaemolyticus	Vibrio	*Moderate*	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
		Low	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
Negative Stool Matrix	Negative	NA	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)
Clostridium difficile	Negative	NA	30/30 100% (88.4-100)	30/30 100% (88.4-100)	30/30 100% (88.4-100)

Clinical Study - Method Comparison

The performance characteristics of the EP test were determined in a multi-site prospective investigation study at seven (7) U.S. institutions by comparing the Verigene EP test results to reference methods, including bacterial culture and automated phenotype identification for the bacterial targets and broth enrichment followed by EIA and PCR amplification/BDS for Stx1/Stx2 typing. The study included the testing of prospectively collected fresh and frozen Cary-Blair specimens and simulated frozen seeded Cary-Blair specimens. Deidentified prospectively-collected specimens were enrolled from individuals receiving routine care requiring enteric pathogens testing. Twelve (12) clinical specimen acquisition sites were used to provide glycerol stocks to seed 408 simulated specimens. These specimens were blinded and shipped to the testing sites and tested alongside prospectively collected specimens.

A total of 1975 specimens were tested with the EP test. Ninety-eight (98) specimens were excluded; 95 prospectively collected and selected specimens and three simulated specimens. Of the remaining 1877 valid specimens, 25 specimens had a final "No Call," resulting in 25 indeterminate specimens. Therefore, a total of 1852 evaluable specimens were used to calculate the performance characteristics for the study. The following table provides a summary of demographic information for 1262 of the 1277 prospectively collected specimens in the valid dataset (age was not recorded for 15 specimens).

Age Range	***No. of Specimens***	***Percentage***
0-1	61	4.8%
>1-5	47	3.7%
>5-12	84	6.7%
>12-21	139	11.0%
>21-65	609	48.3%
>65	322	25.5%
Total	**1262**	**100%**

The table below provides a summary of the clinical performance, stratified by specimen type, of the EP test for the detection of five (5) bacterial targets and Stx1 and Stx2 (n=1852), compared to the above-described reference methods.

	Specimen Type			*n*	*% Agreement (95% CI)*	
					Positive	***Negative***
***Campylobacter* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1243	90.5% 19/21 (69.6-98.8)	98.8% 1207/1222 (98.0-99.3)
			Frozen	34	100% 2/2 (15.8-100)	100% 32/32 (89.1-100)
		Selected		166	97.5% 39/40 (86.8-99.9)	99.2% 125/126 (95.7-100)
		All		1443	95.2% 60/63 (86.7-99.0)	98.8% 1364/1380 (98.1-99.3)
	Simulated			409	98.5% 67/68 (92.1-100)	100% 341/341 (98.9-100)
	All			**1852**	**97.0% 127/131 (92.4-99.2)**	**99.1% 1705/1721 (98.5-99.5)**
***Salmonella* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1243	85.7% 18/21 (63.7-97.0)	99.4% 1215/1222 (98.8-99.8)
			Frozen	34	100% 1/1 (2.5-100)	97.0% 32/33 (84.2-99.9)
		Selected		166	98.2% 53/54 (90.1-100)	99.1% 111/112 (95.1-100)
		All		1443	94.7% 72/76 (87.1-98.6)	99.3% 1358/1367 (98.8-99.7)
	Simulated			409	100% 67/67 (94.6-100)	100% 342/342 (98.9-100)
	All			**1852**	**97.2% 139/143 (93.0-99.2)**	**99.5% 1700/1709 (99.0-99.8)**
***Shigella* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1243	66.7% 2/3 (9.4-99.2)	98.7% 1224/1240 (97.9-99.3)
			Frozen	34	-	97.1% 33/34 (84.7-99.9)
		Selected		166	100% 6/6 (54.1-100)	99.4% 159/160 (96.6-100)
		All		1443	88.9% 8/9 (51.8-99.7)	98.7% 1416/1434 (98.0-99.3)
	Simulated			409	100% 50/50 (92.9-100)	100% 359/359 (99.0-100)
	All			**1852**	**98.3% 58/59 (90.9-100)**	**99.0% 1775/1793 (98.4-99.4)**
***Vibrio* spp.**	Clinical Specimens	Prospectively Collected	Fresh	1242	100% 1/1 (2.5-100)	100% 1242/1242 (99.7-100)
			Frozen	34	100% 1/1 (2.5-100)	100% 33/33 (89.4-100)
		Selected		166	100% 1/1 (2.5-100)	100% 165/165 (97.8-100)
		All		1443	100% 3/3 (29.2-100)	100% 1440/1440 (99.7-100)
	Simulated			409	91.1% 51/56 (80.4-97.0)	99.7% 352/353 (98.4-100)
	All			**1852**	**91.5% 54/59 (81.3-97.2)**	**99.9% 1792/1793 (99.7-100)**
Y. enterocolitica	Clinical Specimens	Prospectively Collected	Fresh	1243	-	100% 1243/1243 (99.7-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		166	100% 1/1 (2.5-100)	100% 165/165 (97.8-100)
		All		1443	100% 1/1 (2.5-100)	100% 1442/1442 (99.7-100)
	Simulated			409	100% 59/59 (93.9-100)	100% 350/350 (99.0-100)
	All			**1852**	**100% 60/60 (94.0-100)**	**100% 1792/1792 (99.8-100)**
Stx1	Clinical Specimens	Prospectively Collected	Fresh	1243	100% 4/4 (39.8-100)	99.7% 1236/1239 (99.2-99.9)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		166	100% 9/9 (66.4-100)	99.4% 156/157 (96.5-100)
		All		1443	100% 13/13 (75.3-100)	99.7% 1426/1430 (99.3-99.9)
	Simulated			409	100% 51/51 (93.0-100)	99.4% 356/358 (98.0-99.9)
	All			**1852**	**100% 64/64 (94.4-100)**	**99.7% 1782/1788 (99.3-99.9)**
Stx2	Clinical Specimens	Prospectively Collected	Fresh	1243	100% 6/6 (54.1-100)	99.8% 1235/1237 (99.4-100)
			Frozen	34	-	100% 34/34 (89.7-100)
		Selected		166	100% 9/9 (66.4-100)	100% 157/157 (97.7-100)
		All		1443	100% 15/15 (78.2-100)	99.9% 1426/1428 (99.5-100)
	Simulated			409	96.7% 58/60 (88.5-99.6)	99.7% 348/349 (98.4-100)
	All			**1852**	**97.3% 73/75 (90.7-99.7)**	**99.8% 1774/1777 (99.5-100)**

Substantial Equivalence

The Verigene® Enteric Pathogen Nucleic Acid Test (EP test) has been shown to be substantially equivalent to the xTAG Gastrointestinal Pathogen Panel (GPP). The EP test has similar intended use and indications, technological characteristics, and performance characteristics. The minor differences between the EP test and its predicate devices raise no new issues of safety or effectiveness. Performance data demonstrate that the EP test is as safe and effective as the predicate device. Thus, the EP test is substantially equivalent to the predicate device.

Similarities		
Element	**New Device:** **Enteric Pathogens Nucleic Acid Test (EP)** **K140083**	**Predicate:** **xTAG® Gastrointestinal Pathogen Panel (GPP)** **K121894**
Intended Use	The Verigene Enteric Pathogens Nucleic Acid Test (EP) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria and genetic virulence markers from liquid or soft stool preserved in Cary-Blair media, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria: • *Campylobacter* Group (comprised of *C. coli*, *C. jejuni*, and *C. lari*) • *Salmonella* species • *Shigella* species (including *S. dysenteriae*, *S. boydii*, *S. sonnei*, and *S. flexneri*) • *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*) • *Yersinia enterocolitica* In addition, EP detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing *E. coli* (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2. EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. EP also aids in	The xTAG® Gastrointestinal Pathogen Panel (GPP) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple viral, parasitic, and bacterial nucleic acids in human stool specimens from individuals with signs and symptoms of infectious colitis or gastroenteritis. The following pathogen types, subtypes and toxin genes are identified using the xTAG® GPP: • *Campylobacter* (*C. jejuni*, *C. coli* and *C. lari* only) • *Clostridium difficile (C. difficile) toxin A/B* • *Cryptosporidium (C. parvum and C. hominis only)* • *Escherichia coli (E. coli) O157* • *Enterotoxigenic Escherichia coli (ETEC) LT/ST* • *Giardia (G. lamblia only - also known as G. intestinalis and G. duodenalis)* • *Norovirus GI/GII* • *Rotavirus A* • *Salmonella* • *Shiga-like Toxin producing E. coli (STEC) stx 1/stx 2* • *Shigella (S. boydii, S. sonnei, S. flexneri and S. dysenteriae)* The detection and identification of specific gastrointestinal microbial nucleic acid from individuals exhibiting signs and symptoms of gastrointestinal infection aids in the diagnosis of gastrointestinal infection when used in conjunction with clinical evaluation, laboratory findings and

Similarities		
Element	**New Device:** **Enteric Pathogens Nucleic Acid Test (EP)** **K140083**	**Predicate:** **xTAG® Gastrointestinal Pathogen Panel (GPP)** **K121894**
	the detection and identification of acute gastroenteritis in the context of outbreaks. Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Yersinia enterocolitica*, *Vibrio* Group and *Shigella* species were primarily established with contrived specimens. Concomitant culture is necessary for organism recovery and further typing of bacterial agents. EP results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative EP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.	epidemiological information. A gastrointestinal microorganism multiplex nucleic acid-based assay also aids in the detection and identification of acute gastroenteritis in the context of outbreaks. xTAG® GPP positive results are presumptive and must be confirmed by FDA cleared tests or other acceptable reference methods. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out coinfection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative xTAG Gastrointestinal Pathogen Panel results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease. xTAG GPP is not intended to monitor or guide treatment for *C. difficile* infections. The xTAG GPP is indicated for use with the Luminex MAGPIX instrument.
Specimen Type	Human Stool sample in Cary-Blair Media	Same
DNA Amplification	PCR	Same
Organisms/NA Targets Detected	*Campylobacter* Group (*C. coli, C. jejuni,* and *C. lari)* *Salmonella* species *Shigella* species (*S. dysenteriae, S. boydii, S. sonnei,* and *S. flexneri)* *Vibrio* Group (comprised of *V. cholerae* and *V. parahaemolyticus*) *Yersinia enterocolitica* Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers	Same with additional analytes (excluding *Vibrio* Group and *Yersinia enterocolitica*).

Differences		
Element	**New Device: Enteric Pathogens Nucleic Acid Test (EP) K140083**	**Predicate: xTAG® Gastrointestinal Pathogen Panel (GPP) K121894**
Time to Result	~ 2 hours	5 hours
Sample prep	On-board, automated NA extraction and amplification	Off-line NA Extraction and amplification
Detection Method	Gold/Silver nanoparticle probe detection of bacterial-specific DNA on complementary oligo- microarray	Specific microbial target or control bead populations coupled to sequences from Universal Array streptavidin, R-phycoerythrin conjugate
Optical Detection	Light scatter	Multi-color fluorescence

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

NOAH LERMER, Ph.D.
DIRECTOR, REGULATORY AFFAIRS
NANOSPHERE, INC.
4088 COMMERCIAL AVENUE
NORTHBROOK IL 60062

June 20, 2014

Re: K140083
Trade/Device Name: Verigene Enteric Pathogen Nucleic Acid Test
Regulation Number: 21 CFR 866.3990
Regulation Name: Gastrointestinal pathogen panel multiplex nucleic acid-based assay system
Regulatory Class: II
Product Code: PCH, PCI, OOI
Dated: May 21, 2014
Received: May 22, 2014

Dear Dr. Lermer:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K140083

Device Name
Verigene® Enteric Pathogens Nucleic Acid Test (EP)

Indications for Use *(Describe)*
The Verigene® Enteric Pathogens Nucleic Acid Test (EP) is a multiplexed, qualitative test for simultaneous detection and identification of common pathogenic enteric bacteria and genetic virulence markers from liquid or soft stool preserved in Cary-Blair media, collected from individuals with signs and symptoms of gastrointestinal infection. The test is performed on the automated Nanosphere Verigene System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific gastrointestinal microbial nucleic acid gene sequences associated with the following pathogenic bacteria:

- Campylobacter Group (comprised of C. coli, C. jejuni, and C. lari)
- Salmonella species
- Shigella species (including S. dysenteriae, S. boydii, S. sonnei, and S. flexneri)
- Vibrio Group (comprised of V. cholerae and V. parahaemolyticus)
- Yersinia enterocolitica

In addition, EP detects the Shiga toxin 1 gene and Shiga toxin 2 gene virulence markers. Shiga toxin producing E. coli (STEC) typically harbor one or both genes that encode for Shiga Toxins 1 and 2.

EP is indicated as an aid in the diagnosis of specific agents of gastrointestinal illness, in conjunction with other clinical, laboratory, and epidemiological information; however, is not to be used to monitor these infections. EP also aids in the detection and identification of acute gastroenteritis in the context of outbreaks.

Due to the limited number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for Yersinia enterocolitica, Vibrio Group and Shigella species were primarily established with contrived specimens.

Concomitant culture is necessary for organism recovery and further typing of bacterial agents.

EP results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Confirmed positive results do not rule out co-infection with other organisms that are not detected by this test, and may not be the sole or definitive cause of patient illness. Negative EP results in the setting of clinical illness compatible with gastroenteritis may be due to infection by pathogens that are not detected by this test or non-infectious causes such as ulcerative colitis, irritable bowel syndrome, or Crohn's disease.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

John Hobson -S
2014.06.19 09:56:26 -04'00'

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